MOSQUITO HOSTS OF ARBOVIRUSES FROM INDIAN RIVER COUNTY, FLORIDA, DURING 1998

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ABSTRACT

Adult mosquitoes were collected for virus isolation from two sites in Indian River County, FL, from May 5 through August 13, 1998 using dry ice-baited CDC light traps (81 trap-nights) and CDC gravid traps (254 trap-nights). A total of 46,150 female mosquitoes (923 mosquito-pools, 50 females/pool) were processed for virus isolation. These females represented 18 species of mosquitoes, with *Culex nigripalpus* comprising 77.4% of all mosquitoes collected, followed by *Aedes infirmatus* (4.9%), *Ae. vexans* (4.0%) and *Cx. erraticus* (2.4%). No St. Louis encephalitis (SLE) and eastern equine encephalitis (EEE) virus isolates were obtained. Keystone (KEY) and Tensaw (TEN) viruses were isolated from *Ae. albopictus* (one isolate of KEY); *Anopheles crucians* (two isolates of TEN); *Cx. nigripalpus* (one isolate of TEN and 2 isolates of KEY); *Coquilletidia perturbans* (two isolates of TEN); and *Wyeomyia vanduzeei* (one isolate of TEN). All isolates were obtained from mosquitoes collected in CDC light traps, except for the KEY virus isolate from *Ae. albopictus*, which was collected in a CDC gravid trap. The isolation of TEN virus from *Wy. vanduzeei* is a first record for Florida.

Key Words: Arbovirues, Tensaw, Keystone, mosquitoes, *Culex nigripalpus*, *Aedes albopictus*, *Coquilletidia perturbans*, *Anopheles crucians*, *Wyeomyia vanduzeei*

RESUMEN

Mosquitos adultos fueron colectados para aislamiento de virus de dos sitios en el Condado de Indian River, FL, desde mayo 5 hasta agosto 13, 1998, usando trampas de luz CDC cebadas con hielo seco (81 noches de trampa) y trampas CDC grávidas (254 noches de trampas). Un total de 46,150 mosquitas (923 grupos de mosquitos, 50 hembras/grupo) fueron procesadas para aislamiento de virus. Estas hembras representaron 18 especies de mosquitos, con *Culex nigripalpus* componiendo 77.4% del total, seguido por *Aedes infirmatus* (4.9%), *Ae. vexann* (4.0%). y *Cx. erraticus* (2.4%). No se obtuvieron virus aislados de encefalitis St. Louis (SLE) o encefalitis oriental equina (EEE). Los virus Keystone (KEY) y Tensaw (TEN), fueron aislados de *Ae. albopictus*, (un aislado de virus KEY); de *Anopheles crucians* (dos aislados de virus TEN); de *Cx. nigripalpus* (un aislado de TEN y dos aislados de KEY); de *Coquillletidia perturbans* (dos aislados de TEN); y de *Wyeomyia vanduzeei* (un aislado de TEN). Todos los aislados fueron obtenidos de mosquitos colectados en trampas de luz CDC, excepto por el aislado del virus KEY de *Ae. albopictus*, el cual fue colectado en una trampa CDC grávida. El aislamiento de virus TEN de *Wy. vanduzeei* es una primera constancia para Florida.

Mosquitoes are vectors and/or hosts of several arboviruses in Florida. These arboviruses include, St. Louis encephalitis (SLE), eastern equine encephalitis (EEE), trivittatus (TVT), Flanders (FLA), Sawgrass (SAW), Tamiami (TAM), Everglades (EVE), Shark River (SR), Jamestown Canyon (JC), Highlands (HJ), Tensaw (TEN), and Keystone (KEY) (Chamberlain et al. 1964; Dow et al. 1964; Wellings et al. 1972; Shroyer 1991; Mitchell et al. 1996). Most of these arboviruses, have been isolated from counties in Florida other than Indian River. Only SLE virus was isolated from mosquitoes in Indian River County in 1990 (Shroyer, 1991). Recently, Mitchell et al. (1996) isolated EEE, EVE, KEY, TEN, TVT, SR, and FLA from mosquitoes associated with waste-tire piles in counties in central and north Florida. In 1997, an above average amount of SLE virus activity in sentinel chickens occurred in Indian River County, Florida, although no human cases were documented there (Day & Stark 2000) with continued SLE virus activity in sentinel chickens in February 1998. There were no virus isolates from the *Culex nigripalpus* Theobald (a proven vector of SLE virus) collected and tested for SLE virus during 1997 in Indian River County (Day & Stark 2000). The purpose of the present study was to isolate and identify arthropod-borne viruses from mosquitoes collected in two different locations in Indian River County during the late spring and early summer of 1998.

Materials and Methods

Mosquito collection sites: Mosquitoes were collected from 2 sites in Indian River County. Site 1 was pine woods with scattered cabbage palm, palmetto, and oak, 25 Km north of the Florida Medical Entomology Laboratory (FMEL), Vero Beach; at this site a sentinel chicken flock was maintained by the Indian River Mosquito Control District as part of its SLE virus surveillance program. The traps were set more than 50 m from the sentinel chicken flock. Site 2 was an oak hammock on the FMEL grounds.

Mosquito collection and handling methods: Mosquitoes were collected twice a week from May 5 through August 13, 1998, in 5 CDC gravid mosquito traps (Reiter 1983) and 1 or 2 dry ice-baited CDC miniature light traps. Mosquitoes from the traps were transported in ice-coolers to the FMEL, where they were separated on a chill table and identified to species; 50 females were then pooled in 2-ml screw-cap cryovials. The cryovials were labeled and frozen at -80%C until they were shipped overnight on dry ice to the CDC laboratory in Fort Collins, Colorado, for virus isolation and identification.

Virus Isolations and *Virus Identification*:

Mosquito pools were triturated in 2 ml of BA-1 diluent by using cold mortars and pestles. BA-1 diluent containing 1× M199 medium with Hanks balanced salt solution (HBSS), 0.05 M Tris pH 7.6, 1% bovine serum albumin, 0.35 gm/L sodium bicarbonate, 100 units/ml penicillin, 100 g/ml streptomycin, 1 g/ml fungizone, and 10 mg/liter phenol red. Suspensions were centrifuged in Eppendorf tubes at 14,000 rpm for 2 min. Supernatants were poured into 1-dram screw-cap vials and stored at -70°C until tested.

Specimens were tested for virus in Vero cell culture grown in 6-well plates. Specimens were inoculated in 0.1-ml quantities in 2 wells each and adsorbed for 1 hr at 37°C; the cells were then overlayed with the first of two nutrient -0.5% agarose overlays. Cell cultures were incubated at 37°C and 3 days later a second agarose overlay containing 1:50,000 neutral red was applied. Cell cultures were returned to the incubator and examined daily thereafter for plaques through day 10 postinoculation.

Virus-positive cell cultures were harvested in 2 ml of BA-1 and frozen at -70°C until they were passed into fluid cultures of Vero cells in 25-cm^2 flasks. When early cytopathic effects (CPE) were noted, infected cells were scraped or trypsinized from the surface of the flask and resuspended in phosphate-buffered saline (PBS), pH 7.4, containing 5% Fetal Bovine Serum (FBS). Twelve-well spot slides were prepared, air-dried, and fixed in cold acetone. These were tested in an indirect fluorescent antibody (IFA) assay (Wulff and Lange 1975) against a battery of hyperimmune grouping ascitic fluids obtained from NIH and CDC. Usually, viral type-specific monoclonal antibodies against common or suspected viruses also were included in the test to definitively identify isolates at this stage.

Virus-positive cell cultures were also characterized by the reverse transcriptase-PCR (RT-PCR) by using flavivirus-consensus, SLE-specific, and bunyavirus serogroup-specific primers (Chang et al. 1994; Kuno et al. 1996). RT-PCR-positive specimens generated by BCS82C and BCS332V primers were genetically sequenced by using an ABI Prism 377 DNA Sequencer (Perkin-Elmer/Applied Biosystems, Foster City, CA) (Kuno et al. 1996). Both strands of the cDNA, located between nucleotide position of 77 to 273 in the small (S) RNA segment, were sequenced and compared with the GenBank data base by using the BLAST search program (http://www.ncbi.nlm.nih.gov/BLAST/). Otherwise, antigenically grouped viral isolates were typed by neutralization (N) assay in Vero cell cultures against reference polyclonal immune reagents prepared against specific members of the antigenic group. Homologous N titers were predetermined for reference reagents used in the identifying N tests.

Results

Totals of 54 light trap and 52 gravid trap were used to collect 81 light and 254 gravid, trap nights, respectively, at the two sites. A total of 94.9% of the female mosquitoes that were tested for viruses were captured in light traps while only 5.1% of the female mosquitoes were collected in gravid traps (Table 1). Mosquitoes in light trap collections were 80.1% *Cx. nigripalpus* with other species comprising less than 5% of the total (Table 1). The main mosquito species in gravid traps were *Aedes albopictus* (Skuse), *Cx. nigripalpus*, *Wyeomyia vanduzeei* Dyar and Knab, and *Wy. mitchellii* (Theobald). All of the *Cx. quinquefasciatus* were collected in gravid traps. (Table 1).

No SLE and EEE virus isolates were obtained from mosquito collected from May 5 through August 13,1998, at the 2 sites. Five species of mosquitoes yielded 9 isolates of arboviruses belonging to 2 antigenic groups, Keystone (KEY) virus of the California antigenic group and Tensaw (TEN) virus of the Bunyamwera antigenic group (Table 2). Four species of mosquitoes yielded 6 isolates of TEN virus: *An. crucians* (two isolates); *Cx. nigripalpus* (one isolate); *Cq. perturbans* (two isolates); and *Wy. vanduzeei* (one isolate). Two species of mosquitoes yielded 3 KEY virus isolates, *Ae. albopictus* (one isolate) and *Cx. nigripalpus* (two isolates). All isolates were obtained from mosquitoes collected in CDC light traps, except for 1 KEY virus isolate, which was recovered from

TABLE 1. MOSQUITOES TESTED FOR THE PRESENCE OF ARBOVIRUSES THAT WERE COLLECTED AT TWO SITES IN INDIAN RIVER COUNTY, FLORIDA, FROM MAY 5 THROUGH AUGUST 13, 1998.

Species	Total tested	Site 1		Site 2		Percentage by species
		Light trap	Gravid trap	Light trap	Gravid trap	
Ae. albopictus	1,000		50	250	700	2.1
Ae. atlanticus	100			100		0.2
Ae. infirmatus	2,250	100		2,150		4.9
Ae. taeniorhynchus	650	150		500		1.4
Ae. vexans	1,850	1,800		50		4.0
An. crucians	750	500		250		1.6
An. quadrimaculatus	50	50				0.1
Cq. perturbans	550	450		100		1.2
Cx. erraticus	1,100	1,100				2.4
Cx. iolambdis	100			100		0.2
Cx. nigripalpus	35,700	26,450	350	8,650	250	77.4
Cx. quinquefasciatus	900		450		450	2.0
Cx. salinarius	400	350			50	0.9
De. cancer	200				200	0.4
Ma. titillans	50	50				0.1
Ps. ferox	50				50	0.1
Wy. mitchellii	100			50	50	0.2
Wy. vanduzeei	350			300	50	0.8
Total	46,150	31,000	850	12,800	1,500	100.0
Percentage by trap and site		67.2	1.8	27.7	3.3	100.0

Ae. albopictus specimens collected in a CDC gravid trap.

The RT-PCR independently confirmed the serological identification that 9 isolates of arboviruses were not SLE or EEE viruses. Nucleotide sequences were obtained from 6 TEN virus isolates and 1 KEY virus isolate. All TEN virus isolates had an identical nucleotide sequence in the S-gene region sequenced. The BLAST search by using GenBank data base indicated that TEN virus isolates shared 95.4%, 95.4% and 93.4% nucleotide identity with Northway, Cache Valley, and Bunyawera viruses, respectively (Table 3). Keystone virus isolate FL-1290 shared 99.5%, 93.4%, and 92.3% nucleotide sequence identity with 1 other KEY virus isolate, Jamestown Canyon and Jerry Slough viruses, respectively.

DISCUSSION

St. Louis encephalitis virus was not isolated from any species of mosquito collected in this study. Similarly, Mitchell, et al. (1996) failed to isolate SLE virus from mosquitoes collected from 36 sites in central and north Florida from April to September, 1993. In 1990, SLE virus was isolated

TABLE 2. VIRUS ISOLATIONS FROM INDIAN RIVER COUNTY, FLORIDA, MOSQUITOES FROM MAY 5 THROUGH AUGUST 13, 1998, AND MINIMUM INFECTION RATES (MIR) BY SITE COLLECTION.

Species	Virus	Site	Isolate number	MIR[1]
Ae. albopictus	KEY	2	FL98-1290	1.0
An. crucians	TEN	2	FL98-1199	2.6
	TEN	2	FL98-1198	2.6
Cx. nigripalpus	TEN	1	FL98-760	0.03
	KEY	1	FL98-5240	0.06
	KEY	1	FL98-5241	0.06
Cq. perturbans	TEN	1	FL98-874	3.6
	TEN	1	FL98-875	3.6
Wy. vanduzeei	TEN	2	FL98-1207	2.9

[1]MIR = Minimum infection rate per 1,000 females tested.

TABLE 3. GENETIC IDENTIFICATION OF VIRUS ISOLATIONS FROM INDIAN RIVER COUNTY, FLORIDA, MOSQUITOES FROM MAY 5 THROUGH AUGUST 13, 1998, BY A BLAST SEARCH OF THE GENBANK DATA BASE.

	Top three scores by the BLAST search (Accession No.; species)		
Strain (FL98-)	X73470; Northway	X73465; Cache Valley	D00353; Bunyamwera
760, 874, 875, 1198, 1199, 1207	95.4 U12801; Keystone	95.4 U12796; Jamestown Canyon	93.4 U12798; Jerry Slough
1290	99.5	93.4	92.3

from *Cx. nigripalpus* in Indian River County during late summer and fall (Shroyer 1991). Interestingly, in the almost 50-year history of the presence of SLE virus in Florida, the virus has only been recovered from *Cx. nigripalpus* during epidemics years (Chamberlain et al. 1964; Dow et al. 1964; Monath and Tsai 1987; Shroyer 1991; Wellings et al. 1972). This could be because mosquitoes were generally collected for virus isolation only during years when epidemics occurred. Furthermore, the minimum infection rates of SLE virus in *Cx. nigripalpus* were low even during epidemics, and ranged from 0.6 to 1.1 per 1000 (Chamberlain et al. 1964; Dow et al. 1964; Shroyer 1991).

Two viruses, KEY and TEN, were isolated from five species of mosquitoes. KEY virus was recovered from *Ae. albopictus* and *Cx. nigripalpus*, and TEN virus from *An. crucians*, *Cx. nigripalpus*, *Cq. perturbans,* and *Wy. vanduzeei*. These two viruses occur in abundance in Florida from April to September each year and have been isolated from several species of mosquitoes, including those collected in our study (Taylor et al. 1971; Wellings et al. 1972; Calisher et al. 1986; Mitchell et al. 1996). Only exception being a (first time) TEN virus isolate from *Wy. vanduzeei*. KEY and TEN viruses also have been isolated from small mammals in Florida. KEY virus was isolated from cotton rats and TEN virus from cotton rats, marsh rabbits, swamp rabbits, and various other small mammals (Taylor et al. 1971; Wellings et al. 1972; Calisher et al. 1986). KEY and TEN viruses are of no public health or veterinary importance.

RT-PCR, followed by nucleotide sequencing of positive specimens, and a GenBank similarity search by using the BLAST search program proved to be effective methods of identifying virus species. By these methods none of the tissue culture- positive specimens contained a detectable level of SLE viral RNA. These results also correlated with serological identification and confirmed that the specimens contained KEY and TEN nucleic acids.

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